No. 2

BIO-RESEARCH CONSULTANTS, INC.
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R E P O R T

to

PHILIP MORRIS INCORPORATED

Report No. 9

Contract C-172

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This document includes
2 pages, ____ tables and
2 ~~figures.~~ graphs

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NITRITES

Studies were continued on the effects of nitrite on the body temperature of mice. When the LD 50 dose of $NaNO_2$ was administered intraperitoneally, there was a marked drop of rectal temperature in both dying and recovering mice. When a dose of 80 mg/kg was used, only a very moderate temperature drop was observed (see Graphs 1 and 2). These results suggest that pharmacological doses of nitrites do not have any effect on rectal temperatures in mice. It was concluded that the measurement of rectal temperatures is inappropriate to study the effects of nitrite.

The following improvements in technique were then considered:

a. Measurement of temperature in subcutaneous tissue of mice. This temperature monitoring should be performed using small thermocouples, now ready for use in our laboratory, and amplifiers have been ordered to increase sensitivity. This approach will permit continuous monitoring of temperature variations of one tenth of a degree without disturbing the animal. This will be applied first to the study of the effects of relatively high doses of nitrites administered intraperitoneally.

b. The use of larger laboratory animals such as rabbits or cats rather than mice. It is expected that it will be easier to monitor pharmacological parameters in these animals, although the smoking machine will still have to be adapted to these larger animals.

ATROPINE STUDIES

Our research objective, as stated before, is to examine whether transfer of atropine by cigarette smoke takes place in mice. It has been decided to monitor this transfer of atropine with pharmacological rather than toxicological parameters.

Dr. Van Dongen suggested the development of a sensitive and quantitative salivation test for mice and has specified the requirements that such a method should meet (Monthly Report No. 8). He then introduced the following experimental approach:

Salivation will be monitored by soaking the saliva of the mouse's mouth into a narrow strip of lithmus paper and by measuring the rate of diffusion of saliva into the paper strip over a period of time. The application of this idea will meet most of the previously stated requirements.

In order to make this idea workable, the following practical problems should be solved:

1) The lithmus paper should be attached to the mouse's mouth;

2) The displacement of the diffusion boundary should be read directly against a reference distance such as a measuring stick;

3) The salivation experiment should be performed on mice under experimental conditions which approximate physiological conditions as closely as possible. The experiment should, therefore, be conducted on animals with a minimum of restraint. It was speculated that this would be accomplished by the development of a "halter" and a "stancion" for attachment of the mouse's head during the actual salivation test.

In the past month, considerable progress has been made in this direction. In total, six versions of mouse halters were developed. In addition, the fourth version of a mouse stancion is in a finishing state. The latest models of both stancion and halter promise to be functional. Some pictures have been taken and will be enclosed in the next monthly report.

If the permanent wearing of halters by mice does not result in health problems and if attachment of a mouse to the stancion allows for adequate and sensitive quantitation of salivation, we will then proceed to make larger numbers of halters and stancions of the best-suited model for studying salivation in a "herd" of mice.

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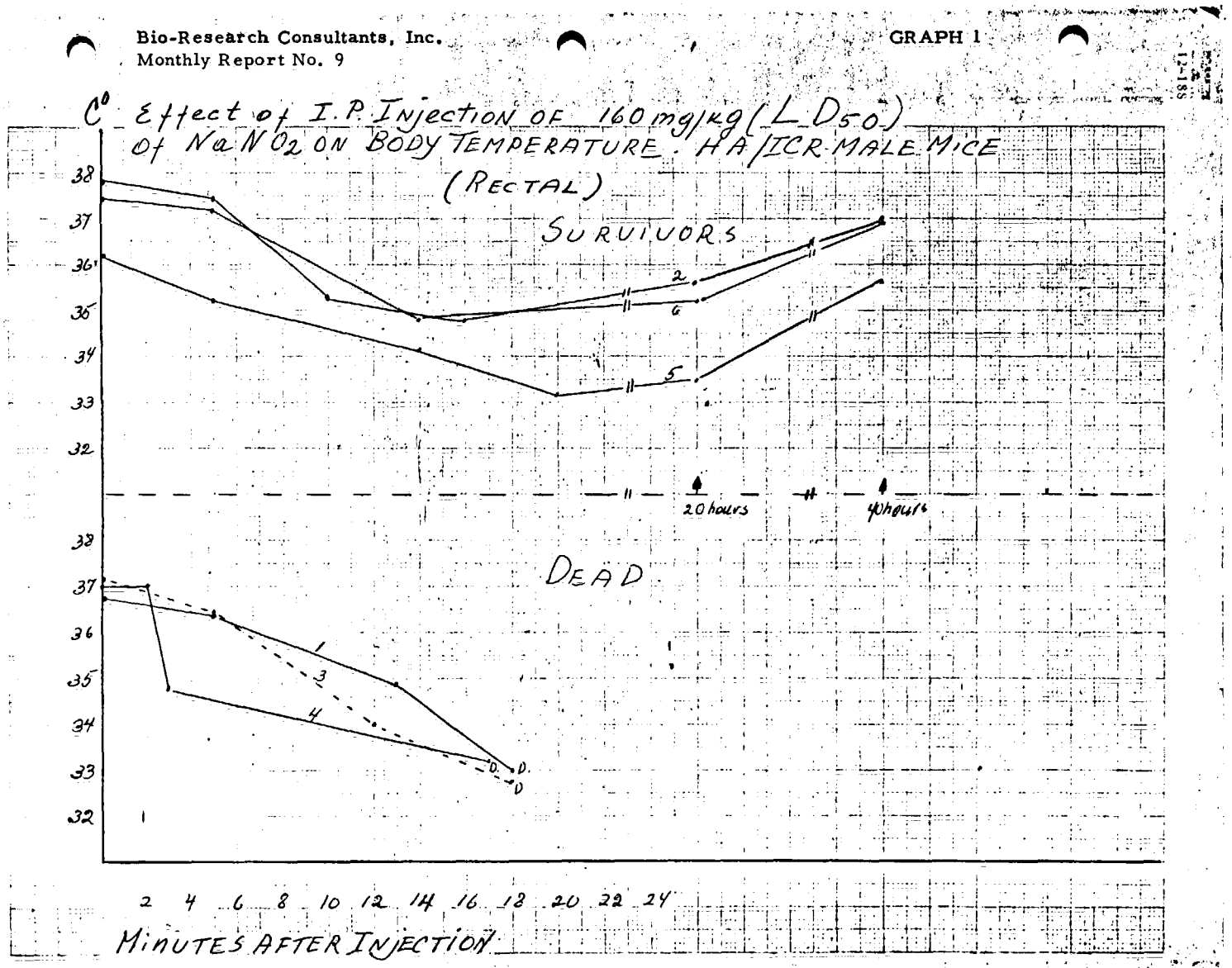
GRAPH 1



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GRAPH 2